

## RESEARCH ARTICLE

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## Antioxidant Activities of *Ficus elastica* Leaves Ethanol Extract and Its Compounds

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**Background:** The excessive free radicals condition called oxidative stress can harmful for the body. To prevent and cure it, the antioxidant agents are required. Nowadays, the natural product extracted from plants have been widely used in folk medicine as antioxidant for the treatment of many diseases. *Ficus elastica* (rubber tree) has some compounds that has several biological activities, *i.e.*, quercitrin, myricitrin, morin, and eleutheroside B. The *F. elastica* works against the free radicals and can be potential as antioxidant agent. The purpose of this study was to evaluate antioxidant properties of *F. elastica* ethanolic extract (FEE), quercitrin, myricitrin, morin, and eleutheroside B.

**Materials and Methods:** The antioxidant activities of FEE and standard compounds were evaluated by free radical-scavenging activity of 2,2-diphenyl-1-picrylhydrazil (DPPH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), 2,2'-azinobis-(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) activities using spectrophotometry method.

**Results:** FEE has the lowest of DPPH scavenging activity (IC<sub>50</sub>=13.82 µg/mL) than other compounds. In ABTS scavenging activity, FEE has moderate activity with IC<sub>50</sub> value 23.29 µg/mL. In FRAP activity, FEE has moderate activity with value 241.58 µM Fe(II)/µg, while in H<sub>2</sub>O<sub>2</sub> scavenging activity, FEE also show moderate activity with IC<sub>50</sub>=83.97 µg/mL compared to other compounds.

**Conclusion:** In summary, FEE and the pure compounds (quercitrin, myricitrin, morin, and eleutheroside B) have potential as antioxidant agent.

**Keywords:** free radical, morin, myricitrin, quercitrin, rubber tree, scavenging activities

### Introduction

Reactive oxygen species (ROS) are highly reactive molecules that may leading to tissues damage via several different cellular molecular pathways.<sup>1,2</sup> The excessive

of free radicals or ROS will create imbalances between free radical molecules and antioxidants and may cause implicated in several metabolic diseases that include heart diseases, diabetes mellitus, skin aging, arthritis, cancer, *etc.*<sup>3</sup> Indeed, such mechanisms could receive substantial relief by

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antioxidants. Antioxidants are widely used as ingredients in dietary supplements for health body. The low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress which contributed to many human diseases.<sup>4</sup>

The World Health Organization has realization of the importance of natural products as a therapeutic ingredient alternative which would be able affordable to majority of the world population.<sup>5</sup> In addition, numerous studies have demonstrated the beneficial effects of natural compounds and antioxidants potential in some medicinal plants.<sup>6-8</sup> Medicinal plants has advantages and can be alternative source of new chemical with potential therapeutic as antioxidant.

*Ficus elastica*, commonly known as rubber tree, is an important medicinal plant belonging to the *Moraceae* family.<sup>9</sup> *F. elastica* plants are has been widely planted throughout Asia<sup>10</sup> possesses pharmacological properties such as antioxidant<sup>9</sup>, anti-inflammatory, and anticancer<sup>11</sup>. *Ficus* species are reported to be very rich in flavonoids, essential oils, anthocyanins tannins and others phenolic constituents.<sup>12</sup> *F. elastica* contain compounds such as rutin, luteolin, coumarins, quercitrin, kaempferin, myricitrin, syringin (eleutheroside B), morin.<sup>9,13</sup> The *F. elastica* leaves extract has antioxidant activity for the treatment of skin infections and skin allergies.<sup>9</sup> Hence, the present study has aim to determined the medicinal properties of *F. elastica* for their potential as antioxidant due to 2,2-Diphenyl-1-picrylhydrazil (DPPH) scavenging activity, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity, 2,2'-Azinobis-(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) scavenging activity and ferric reducing antioxidant power assay (FRAP) activity compared to standard compounds (quercitrin, myricetin, morin, and eleutheroside B).

## Materials and methods

### Preparation of *F. elastica* Extract

The leaves of *F. elastica* L. were obtained from Raya Village, Karo, Sumatera Utara, Indonesia. Samples were identified by taxonomist of School of Life Science and Technology, Institut Teknologi Bandung, Bandung, Indonesia (No. Specimen: 0140718-A017). About 195 g of dried *F. elastica* leaves were mashed, and 2400 mL 70% ethanol solvents was used for extraction and every 24 h, the ethanol filtrate were collected until the filtrate became colourless. This maceration method was using evaporator with 50°C temperature to obtain extract. As much as 9.42

g of *F. elastica* extract was stored at -20°C.<sup>8,14</sup> Quercitrin (Catalogue #BP1192, Chengdu Biopurify Phytochemicals Ltd., Chengdu, China), morin (Catalogue #BP0959, Chengdu Biopurify Phytochemicals), myricitrin (Catalogue #BP0971, Chengdu Biopurify Phytochemicals), and eleutheroside B (Catalogue #BP0525, Chengdu Biopurify Phytochemicals) were used as standard compounds. In this study, all the samples were dissolved in dimethyl sulfoxide (DMSO) 10% before the used.

### DPPH Free Radical Scavenging Assay

The 50 µL of samples (*F. elastica* extract, quercitrin, myricitrin, morin, and eleutheroside B) in various concentration (0.98; 1.95; 3.91; 7.82; 15.63; 31.26 µg/mL) were introduced in a 96-well microplate. Two hundred µL of DPPH solution (Catalogue #D9132, SigmaAldrich, Missouri, USA) was added and then incubated for 30 min at a room temperature in the dark room. Moreover, the absorbance at 517 nm wave length was read using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Scientific, Massachusetts, USA).<sup>7,8,15</sup> The experiment was carried out in triplicate and DPPH scavenging activity was calculated using the equation:

$$\% \text{ scavenging activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

### ABTS Scavenging Activity Assay

ABTS assay of FEE, quercitrin, morin, myricitrin, and eleutheroside B were conducted based on cited methods with modification. Around 14 mM of ABTS and 4.9 mM of potassium persulfate (Catalogue #EM105091, Merck, New Jersey, USA) was reacted in dark room, for 16 h at room temperature. The mixture solution was mixed with 5.5 mM phosphate-buffered saline (PBS) until the absorbance value reached 0.70±0.02 at 745 nm wavelength. Two µL of sample in various concentration (1.56; 3.13; 6.25; 12.50; 25.00; 50.00 µg/mL) were added to each well at 96-well microplate, 198 µL ABTS solution was added and then incubated at 30°C, for 6 min. The absorbance was measured at 745 nm using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Scientific).<sup>14-16</sup>

The analysis was carried out in triplicates. The ABTS reducing activity was calculated with the formula:

$$\% \text{ reducing activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

### FRAP Asssay

The FRAP was done using modified method.<sup>8,14,16</sup> As much as 7.50  $\mu$ L samples with various concentration and 142.50  $\mu$ L of FRAP reagent was introduced into 96-well plate. And then incubated for 30 min at 37°C. Briefly, the absorbance was measured with a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Scientific) at 593 nm.

### $H_2O_2$ Scavenging Activity

Sixty  $\mu$ L of *F. elastica* extract, quercitrin, myricetin, morin, and eleutheroside B was added to 96-well plate and in well blanks. Briefly, 12  $\mu$ L of ferrous ammonium sulfate (1 mM) (Catalogue #215406, Sigma Aldrich) was added to the well control and sample wells. Sixty-three of DMSO (Catalogue #1.02952.1000, Supelco, Missouri, USA) was added in the well control and 90  $\mu$ L in the well blank, and 3  $\mu$ L of  $H_2O_2$  (5 mM) (Catalogue #1.08597.1000, Merck) added to the well-sample. Then, 3  $\mu$ L, 5 mM of  $H_2O_2$  were added into 96-well plates, and then incubated for 5 min at room temperature, in a dark room. The mixture of sample and blank was added 1,10-phenanthroline (Catalogue #131377, Sigma Aldrich) for around 75  $\mu$ L, then incubated again for 10 min as before. The absorbance value of control and sample also was measured at 510 nm using a microplate reader.<sup>14-16</sup> The experiment was carried out in triplicates. The percentage inhibition of  $H_2O_2$  scavenging activity using the following equation:

$$\% \text{ scavenging activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

## Results

### DPPH Free Radical Scavenging Activity

Based on the results, the perctange of DPPH scavenging activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B could be seen in Figure 1. FEE had the lowest activity in the highest concentration (31.26  $\mu$ g/mL) with value 62.52 $\pm$ 0.66% compared to other compounds (FEE < eleutheroside B < morin < quercitrin < myricitrin). Myricitrin had the highest activity with value 96.22 $\pm$ 1.64%. This indicated that FEE has low antioxidant activity through DPPH scavenging activity.

Table 1 showed that the IC<sub>50</sub> value of DPPH scavenging activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B. FEE showed the highest IC<sub>50</sub> value (13.82 $\pm$ 0.51  $\mu$ g/mL) compared to other compounds, this

**Table 1. The IC<sub>50</sub> of DPPH free radical scavenging of FEE, quercitrin, morin, myricitrin, and eleutheroside B.**

Sample	IC <sub>50</sub> value ( $\mu$ g/mL)
FEE	13.82 $\pm$ 0.51
Quercitrin	1.39 $\pm$ 0.16
Morin	0.88 $\pm$ 0.51
Myricitrin	0.37 $\pm$ 0.45
Eleutheroside B	11.01 $\pm$ 0.15

indicated that FEE has the lowest in DPPH scavenging activity.

### ABTS Scavenging Activity

ABTS scavenging activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B were showed in Figure 2. In Figure 2, FEE has moderate in ABTS scavenging activity (94.46 $\pm$ 0.56%) compared to myricitrin (98.15 $\pm$ 0.14%), quercitrin (95.97 $\pm$ 0.66%), morin (93.56 $\pm$ 0.63%), and eleutheroside B (51.28 $\pm$ 0.95%).

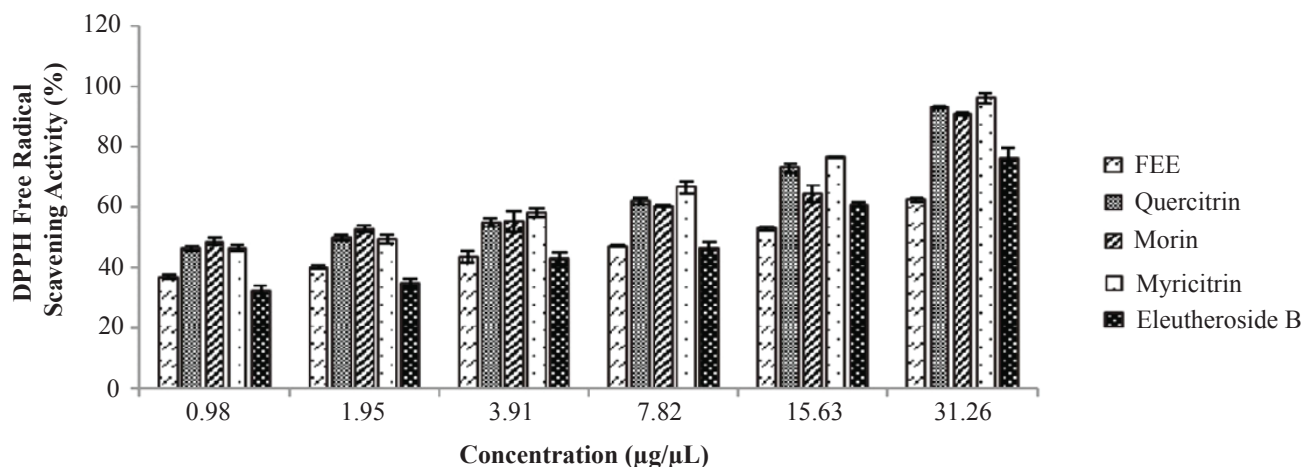
In Table 2, Morin has the highest ABTS scavenging activity (8.11 $\pm$ 0.60  $\mu$ g/mL) compared to FEE and other compounds, while FEE had moderate activity (23.29 $\pm$ 0.07  $\mu$ g/mL) compared to compounds. However, morin has the highest ABTS scavenging activity compared to FEE and other compounds.

### FRAP-reducing Activity

Figure 3 show FRAP-reducing activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B. FEE show the moderate FRAP-reducing activity compared to eleutheroside B, myricitrin, quercitrin, and morin in the highest concentration (50.00  $\mu$ g/mL) (FEE : 241.58 $\pm$ 0.52

**Table 2. The IC<sub>50</sub> of ABTS reducing activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B.**

Sample	IC <sub>50</sub> value ( $\mu$ g/mL)
FEE	23.29 $\pm$ 0.07
Quercitrin	15.48 $\pm$ 0.22
Morin	8.11 $\pm$ 0.60
Myricitrin	8.26 $\pm$ 0.68
Eleutheroside B	46.26 $\pm$ 1.06



**Figure 1. DPPH free radical scavenging activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B.** FEE, quercitrin, morin, myricitrin and eleutheroside B were diluted in DMSO 10% to reach the final concentration of 0.98; 1.95; 3.91; 7.82; 15.63; 31.26 µg/µL.

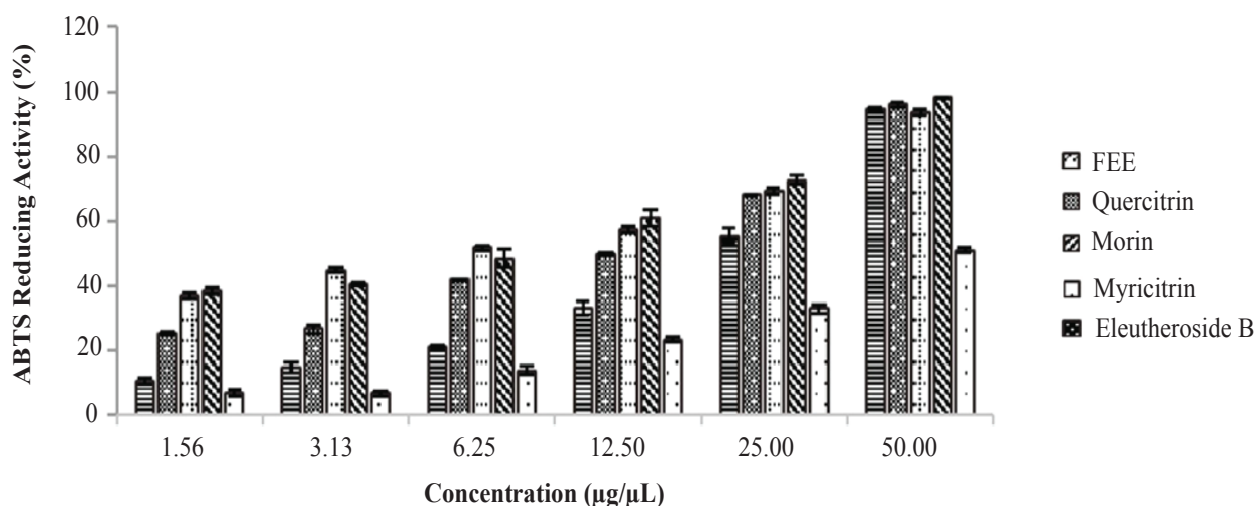
µM Fe(II)/µg, eleutheroside B: 117.08±27.35 µM Fe(II)/µg, myricitrin: 456.00±13.43 µM Fe(II)/µg, quercitrin: 487.58±5.59 µM Fe(II)/µg, morin: 496.58±9.25 µM Fe(II)/µg). Therefore, FEE has moderate antioxidant activity and not higher compared to morin compound.

#### *H<sub>2</sub>O<sub>2</sub> Scavenging Activity*

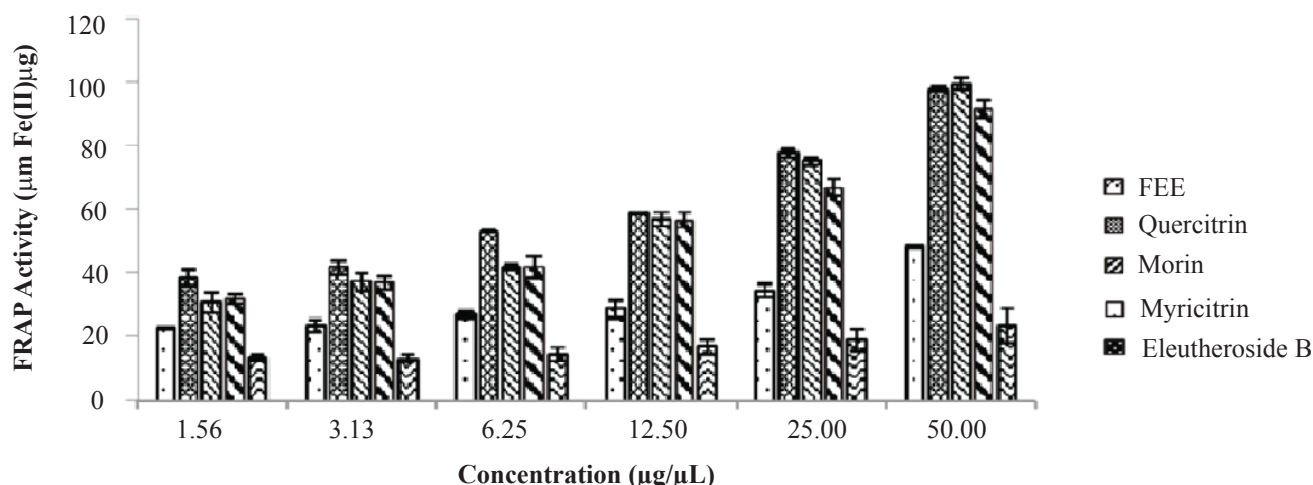
Figure 4 show the H<sub>2</sub>O<sub>2</sub> scavenging activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B. FEE show moderate of H<sub>2</sub>O<sub>2</sub> scavenging activity compared to

the others in the highest concentration 125.00 µg/mL (FEE: 61.55±2.84%, eleutheroside B: 38.49±0.27%, quercitrin: 61.32±0.16%, morin: 64.92±1.45%, and myricitrin: 75.07±3.96%. That indicated FEE has antioxidant activity but not higher than myricitrin.

As shown in Table 3, FEE show the moderate value (83.97±2.23 µg/mL) compared to eleutheroside B, morin, myricitrin, and quercitrin. That show FEE has moderate antioxidant activity but not higher than quercitrin (49.11±5.12 µg/mL).



**Figure 2. AABTS reducing activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B.** FEE, quercitrin, morin, myricitrin, and eleutheroside B were diluted in DMSO 10% to reach the final concentration of 1.56; 3.13; 6.25; 12.50; 25.00; 50.00 µg/µL.



**Figure 3. FRAP activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B.** FEE, quercitrin, morin, myricitrin, and eleutheroside B were diluted in DMSO 10% to reach the final concentration of 1.56; 3.13; 6.25; 12.50; 25.00; 50.00 µg/µL.

## Discussion

*F. elastica* from Moraceae family has been known as antioxidant properties. Ficus leaves has the highest amount of bioactive compound compared to peels and pulp.<sup>17</sup> *F. elastica* has many compounds such as rutin, luteolin, coumarins, quercitrin, kaempferin, myricitrin, syringin (eleutheroside B), and morin.<sup>9,13</sup> Based on phytochemical analysis, the methabolic extract of *F. elastica* leaves showed the presence of carbohydrates, tannins, phytosterols, phenolics, and flavonoids. *F. elastica* extract also has high quantity of total phenolic and flavonoid with each value 64.005 mg GAE/gm and 43.003 mg QE/gm.<sup>18</sup> Antioxidant activities of *F. elastica* and its compounds was carried out in this study through DPPH, H<sub>2</sub>O<sub>2</sub> radicals scavenging activities, ABTS scavenging activity, FRAP activity. These

antioxidant methods are used in this study because has some advantages such as simple, cheap, user friendly, and show the representative data.<sup>19</sup>

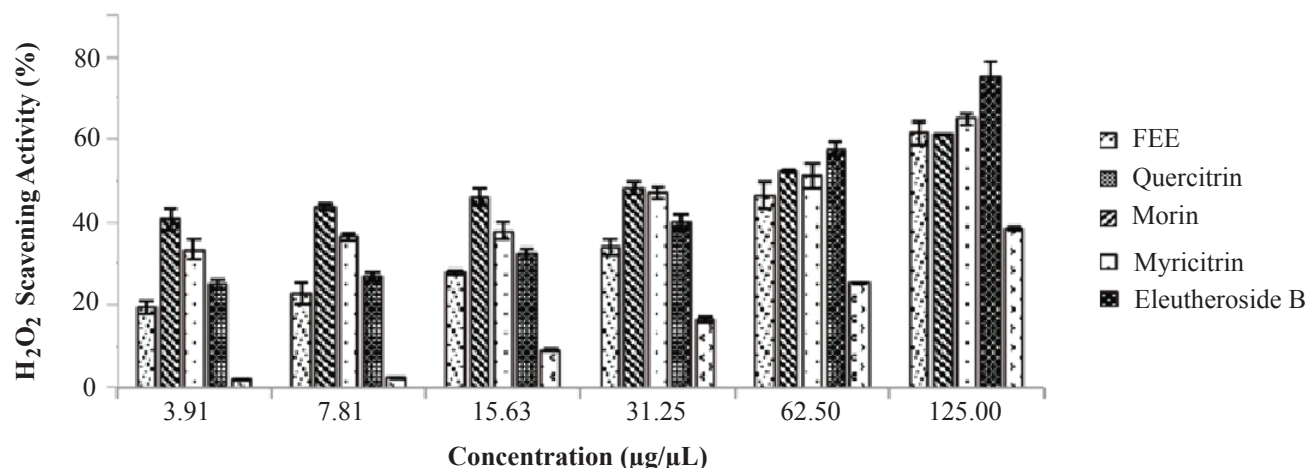
Antioxidant activity in the sample was detected by change colour of DPPH to dark purple DPPH after receiving protons from antioxidants and reducing the color of the protonated DPPH molecules to 1,1 diphenyl-2-picrylhydrazine which was yellowish or pale yellow.<sup>20</sup> The leaves methanol extract of some species Ficus has DPPH scavenging activity.<sup>21</sup> These results indicate that the methanol extracts have scavenging free radicals activities and can be related to the high phenolic compound present.<sup>3</sup> In the present study, quercitrin has moderate activity while myricitrin has the highest DPPH scavenging activity (Table 1). This result in line with other study which state that quercitrin has moderate antioxidant activity because has DPPH activity value 65.05% and has IC<sub>50</sub> value 11.17 µM.<sup>22</sup> In other study, the methanolic extract of *F. elastica* leaves has potent as antioxidant because has the highest in DPPH scavenging activity (IC<sub>50</sub>=15.40 µg/mL) compared to methanolic extract of *F. elastica* branches (IC<sub>50</sub>=26.90 µg/mL).<sup>23</sup> Myricitrin or myricetin-3-O-a-rhamnoside is a phenolic compound which exhibited antioxidant activity due to DPPH scavenging activity (IC<sub>50</sub>=1.31±0.19 µg/mL) and H<sub>2</sub>O<sub>2</sub> scavenging activity (IC<sub>50</sub>=28.46±0.67 µg/mL) compared to silymarin.<sup>24</sup>

The present study show that morin has the highest in DPPH and ABTS scavenging activities compared to other compounds (Table 1 and 2). The methanol extract

**Table 3. The IC<sub>50</sub> value of H<sub>2</sub>O<sub>2</sub> scavenging activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B.**

Sample	IC <sub>50</sub> value (µg/mL)
FEE	83.97±2.23
Quercitrin	49.11±5.12
Morin	60.29±3.62
Myricitrin	58.04±1.84
Eleutheroside B	155.47±1.27





**Figure 4.**  $\text{H}_2\text{O}_2$  scavenging activity of FEE, quercitrin, morin, myricitrin, and eleutheroside B. FEE, quercitrin, morin, myricitrin, and eleutheroside B were diluted in DMSO 10% to reach the final concentration of 3.91; 7.81; 15.63; 31.25; 62.50; 125.00  $\mu\text{g}/\mu\text{L}$ .

from bark, fruits, and leaves *F. microcarpa* exhibited strong as antioxidant through DPPH and ABTS scavenging activities.<sup>17</sup> The major phenolic compounds in medicinal plants exhibit stronger antioxidant activity compared to vegetables and fruits.<sup>25</sup> In other study show that *Ficus glomerate* also has antioxidant activity due to the presence of flavonoids and phenolics in the extracts.<sup>17</sup> Morin is a kind of flavonoid compound belonging to the phenolic group which are one of the secondary metabolites of plants and it has antioxidant activity.<sup>26</sup> Morin is a member flavonols that has ability of reducing oxidative stress and enhancing antioxidant status in hyperammonemic rats.<sup>27</sup> Flavonoid has antioxidant activity through their redox properties and act as reducing agents, singlet oxygen quenchers, hydrogen donors, and have a metallic chelating potential.<sup>25,28</sup>

In FRAP activity, FEE has moderate activity ( $241.58 \pm 0.52 \mu\text{M Fe(II)}/\mu\text{g}$ ) compared other compounds and morin has the highest FRAP activity (Figure 3). This result supported by other study that morin show good antioxidant activity in DPPH scavenging activity with  $\text{IC}_{50}$  value  $59.00 \mu\text{M}$ , and FRAP activity with  $\text{IC}_{50}$  value  $38.00 \mu\text{M}$ .<sup>29</sup> The high total phenolic and flavonoid content in leaf galls of *F. glomerate* methanolic extract may be contributed in ferric reducing power activity. However, the methanolic extract has higher antioxidant potential than aqueous extract.<sup>3</sup> Number and position of hydroxyl group of phenolic compounds also attributed in their antioxidant activity.<sup>3</sup>

In the present study we using ethanol as solvent of *F. elastica* extract. The extracts using a high polarity

solvent (methanol or ethanol) has more effective as radical scavengers.<sup>3</sup> Based on the results, quercitrin as flavonoid compound has the highest  $\text{H}_2\text{O}_2$  scavenging activity ( $\text{IC}_{50} = 49.11 \pm 5.12 \mu\text{g}/\text{mL}$ ) (Table 3), it show that the  $\text{H}_2\text{O}_2$  scavenging activity possessed by quercitrin. In other study was reported that quercitrin also has the highest antioxidant activity (Trolox Equivalent) while myricitrin has the highest in antioxidant activity through reducing capacity.<sup>9</sup> *Ficus carica* leaves also has antioxidant activity due to the presence of steroids and flavonoids.<sup>30</sup> However, *F. elastica* and its compounds can acts as antioxidant agent.

## Conclusion

In this study, we summarize that myricitrin has the highest antioxidant activity through DPPH scavenging activity. In ABTS and  $\text{H}_2\text{O}_2$  scavenging, morin has the highest activities. while quercitrin show the highest antioxidant activity through FRAP activity than *F. elastica* extract.

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